REMARKS

Status of the Claims

Claim 16 has been canceled as being substantially duplicative of claim 13. Claims 1-9, 11, 12, 15, 18, and 21 have been amended as described below. New claims 24-33 have been added. Support for these amendments and the new claims can be found throughout the specification. Therefore, no new matter has been added by way of claim amendment. Entry of these claim amendments into the above-referenced application is respectfully requested.

Specifically, claims 1, 8, 12, 15, and 21 have been amended to recite a nucleotide sequence that is antisense to "the full-length" sequence of SEQ ID NO. 1. Support for this amendment resides in the specification, for example, at page 9, lines 16-18; at page 10, line 28, through page 11, line 4; and at page 12, lines 23-30. Claims 1, 12, and 21 have also been amended to insert the word "nucleotide" into element (a) to clarify that the sequence is a nucleotide sequence as recited in the preamble, and to correct for an error in punctuation in element (a). Claims reciting the phrase "chimeric gene" (i.e., claims 2-9, and 11) or "gene" (claim 12) have been amended to adopt the Examiner's suggestion to recite a "chimeric nucleic acid sequence" or "nucleic acid sequence," respectively. Claim 6 has been amended to depend from claim 4, which is directed to a specific chimeric nucleic acid sequence. Claim 18 has been amended to recite claim dependency on claim 12. No new matter is added by way of these claim amendments.

Further, independent claims 1, 12, and 21 have been amended to remove reference to elements (d)-(g), which were directed to SEQ ID NO. 3, sequences encoding the C-terminal domain of the poly ADP-ribose polymerase set forth in SEQ ID NO. 2 or SEQ ID NO. 4, and sequences that recite hybridization language.

New claim 24 is directed to an isolated DNA molecule comprising a nucleotide sequence that has at least 90% sequence identity with SEQ ID NO. 1. The nucleotide sequence encodes a polypeptide having poly ADP-ribose polymerase activity and which has at least two functional zinc fingers. Support for this claim resides in the

specification, for example, at page 2, lines 21-22, and page 24, lines 12-13. Claim 25 is directed to a specific embodiment of claim 24, where the nucleotide sequence encoding the polypeptide comprises SEQ ID NO. 5, which sets forth that portion of the N-terminal region of the poly ADP-ribose polymerase of SEQ ID NO. 2 that comprises the two zinc fingers of this novel maize protein. Support for this claim resides in the specification, for example, at page 2, lines 17-18. Claims 26-32 are directed to chimeric nucleic acid sequences, vectors, transformed plant cells, and transformed plants comprising the sequence of claim 24. Claim 33 is directed to a method of using the sequence of claim 24 to modulate the metabolic state of a plant cell. Support for these claims resides throughout the specification and in the original claims. No new matter is added by way of presentation of these claims.

Claims 1-15 and 17-33 are pending in the application. Entry of these amendments into the above-referenced application is respectfully requested.

The Examiner's comments are addressed below in the order set forth in the Office Action.

The Rejection of the Claims Under 35 U.S.C. §112, First Paragraph, Should Be Withdrawn

Claims 1, 8, 12, and 21 are rejected under 35 U.S.C. §112 as lacking adequate written description with regard to antisense sequences. This rejection is respectfully traversed as applied to the amended claims.

Amended claims 1, 8, 12, and 21 specifically recite a nucleotide sequence that is antisense to the full-length sequence of SEQ ID NO. 1. Having disclosed the sense sequence as SEQ ID NO. 1, one of skill in the art would readily recognize that Applicants were in possession of a nucleotide sequence that is antisense to the full-length sequence of SEQ ID NO. 1 at the time this application was filed. Further, the specification provides a detailed disclosure of plant transformation techniques and suitable promoters for driving expression of nucleotide sequences that are antisense to the sequence of SEQ ID NO. 1. See, for example, the specification at page 15, lines 1-30, and page 12, lines 1-

22. Given this disclosure, one of skill in the art would readily recognize that Applicants were in possession of a method for modulating the metabolic state of a plant cell using the claimed nucleotide sequence that is antisense to the full-length sequence of SEQ ID NO. 1.

The Office Action states that Applicants have "failed to provide structures of the antisense sequences claimed" and have failed to disclose "any relevant examples of antisense molecules" (Office Action mailed March 26, 2002 (hereinafter "the Office Action), at page 3, lines 8-9). The Office Action further states that Applicants have "also failed to describe a correlation between the antisense sequences encompassed by the breadth of the instant claims and their function" (see the Office Action at page 3, lines 10-11), and have "not shown any antisense sequences which would modulate the activity of the polymerase in the cell" (see the Office Action at page 3, lines 17-18). Applicants respectfully disagree.

In order to satisfy the written description requirement, the specification must reasonably convey to persons skilled in the art that, as of the filing date thereof, the inventor had possession of the subject matter later claimed by him. *See Ex part Parks*, 30 USPQ 1234, 1236-37 (B.P.A.I. 1993). The Examiner has the burden of presenting evidence or reasons why persons skilled in the art would not recognize in the specification disclosure a description of the invention defined by the claims. *Ex parte Sorenson*, 3USPQ2D 1462, 1463 (B.P.A.I. 1987).

A description can be by structure, formula, chemical name, or physical properties. *See Ex parte Maizel*, 27 USPQ2d 1662, 1669 (B.P.A.I. 1992), *citing Amgen v. Chugai*, 927 F.2d 1200, 1206 (Fed. Cir. 1991). A genus of DNAs may therefore be described by means of a recitation of a representative number of DNAs, defined by nucleotide sequence, falling within the scope of the genus, *or* by means of a recitation of structural features common to the genus, which features constitute a substantial portion of the genus. *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1569 (Fed. Cir. 1997); *see also* Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement, 66 Fed. Reg. 1099, 1106 (2000). An Applicant may also rely upon functional characteristics in the description, provided

there is a correlation between the function and structure of the claimed invention. *Id.*, *citing Lilly* at 1568.

In the present case, Applicants have presented information sufficient to satisfy the written description requirement as detailed above. Specifically, Applicants have provided the nucleotide sequence of SEQ ID NO. 1 of the invention. This sequence is sufficient to provide adequate written description for this molecule itself and also for a nucleotide sequence that is antisense to the full-length sequence of SEQ ID NO. 1 as recited in these claims. As noted above, the claimed antisense sequence has a definitive structure in that it is antisense to the full-length sequence of SEQ ID NO. 1. Furthermore, the correlation between sequences that are antisense to poly ADP-ribose polymerase coding sequences and their ability to inhibit expression of the encoded protein, thereby modulating the metabolic state of a plant cell, is well known in the art. Thus, those skilled in the art have successfully used antisense suppression, as well as over-expression, of poly ADP-ribose polymerase nucleic acid sequences to modulate the activity of the class of plant poly ADP-ribose polymerase enzymes *in vivo*. See, Amor-Yehudit *et al.* (1998) *FEBS-Letters* 440(1-2):1-7; Simbulan-Rosenthal *et al.* (1996) *Biochemistry* 35(36):11622-11633; Simbulan-Rosenthal *et al.* (1996) *Proc. Nucleic Acid Res. Mol. Biol.* 55:135-156; Simbulan-Rosenthal *et al.* (1998) *Biochemistry* 37(26):9363-9370; Simbulan-Rosenthal *et al.* (1999) *Biochemistry* 193(1-2):137-148. Copies of the Simbulan-Rosenthal *et al.* (1996) articles and the Amor-Yehudit *et al.* (1998) article were submitted as cite nos. 17, 18, and 20 in the Information Disclosure Statement filed June 18, 1999. Abstracts of the Simbulan-Rosenthal *et al.* 1998 and 1999 articles are filed concurrently herewith as items 1 and 2 of Appendix A. Thus, Applicants respectfully submit that one of skill in the art equipped with the disclosure in Applicants' specification would readily recognize that the composition and method claims directed to a nucleotide sequence that is antisense to the full-length sequence of SEQ ID NO. 1 were in Applicants' possession at the time this application was filed.

The Examiner refers to a need for a demonstration that the claimed antisense molecules would modulate the activity of the poly ADP-ribose polymerase encoded by SEQ ID NO. 1 in order to conclude that the claimed antisense sequences were indeed in

Applicants' possession (the Office Action, at page 3, lines 17-18). However, this requirement is incommensurate with the actual written description requirement described above. Applying the correct standard, Applicants have indeed satisfied the written description requirement. Therefore, the rejection under 35 U.S.C. §112, first paragraph, should be withdrawn.

Claims 1-23 are rejected under 35 U.S.C. §112, first paragraph, for lack of written description with regard to recitation of the phrase "chimeric gene." The original claims that recite this phrase have been amended to adopt the Examiner's suggestion. Thus, claims 2-9 and 11 (reciting the phrase "chimeric gene") and claim 12 (reciting "gene") have been amended to adopt the Examiner's suggestion to recite a "chimeric nucleic acid sequence" or "nucleic acid sequence," respectively. Thus, the rejection is obviated by these claim amendments, and therefore should be withdrawn.

The Rejection of the Claims Under 35 U.S.C. §112, Second Paragraph, Should Be Withdrawn

Claims 1, 12, and 21 are rejected under 35 U.S.C. §112, second paragraph, for recitation of hybridization language without mention of specific hybridization conditions. These claims have been amended to remove reference to sequences that hybridize, thereby obviating this rejection. Thus, this rejection should be withdrawn.

The Rejection of the Claims Under 35 U.S.C. §102 Should Be Withdrawn

Claim 1 is rejected under 35 U.S.C. §102(a) as being anticipated by by Accession No. AJ222589 (Babiyachuk *et al.*, 19 November 1997). Claim 1 has been amended to remove reference to SEQ ID NO. 3 and SEQ ID NO. 4. This rejection is respectfully traversed as applied to the amended claims.

The Office Action states that SEQ ID NO. 1 and SEQ ID NO. 2 respectively share 97% and 95% identity to Accession No. AJ222589 (Babiyachuk *et al.*, 19 November 1997). In response, Applicants respectfully submit that SEQ ID NO. 1 and SEQ ID NO. 2 are not anticipated by Babiyachuk *et al.* because they are not identical to Accession No.

AJ222589. These sequences share similarity with the putative maize poly ADP-ribose polymerase sequences disclosed by Babiyachuk *et al.* However, SEQ ID NO. 1 encodes the full-length maize poly ADP-ribose polymerase protein, which consists of 980 amino acid residues, while the nucleotide sequence disclosed by Babiyachuk *et al.* encodes a partial-length maize poly ADP-ribose polymerase protein that consists of 969 amino acid residues. The novel nucleotide sequence differs by 33 nucleotides, which encode for 11 contiguous amino acids, the first 2 of which reside within the first zinc finger of the maize poly ADP-ribose polymerase protein. These 11 amino acids are set forth as residues 54-64 of SEQ ID NO.2. These sequences are novel, and hence the rejection under 35 U.S.C. §102 should be withdrawn.

The Rejection of the Claims Under 35 U.S.C. §103 Should Be Withdrawn

Claims 1-23 are rejected under 35 U.S.C. §103 as being unpatentable over Accession No. AJ222589 as applied to claim 1 above in view of DeBlock (WO 97/06267). Independent claims 1, 12, and 21 have been amended to remove reference to SEQ ID NO.3 and SEQ ID NO.4. This rejection is respectfully traversed as applied to SEQ ID NO.1 and SEQ ID NO.2 recited in the amended claims.

The amended claims are directed to a chimeric nucleic acid sequence (claims 2, 3, 4, and 8), a transformation vector (claims 5 and 9), transformed plant cells, plants, and seeds (claims 6, 7, and 10-15 and 17-20), and a method for modulating the metabolic state of a plant cell (claims 21-23). All of these claims require knowledge or use of the novel nucleotide sequence set forth in claim 1 and SEQ ID NO. 1.

Prior to Applicants' disclosure, one skilled in the art would not have known that the nucleotide sequence disclosed by Babiyachuk *et al.* encoded a partial-length maize poly ADP-ribose polymerase protein. Furthermore, based on either Babiyachuk *et al.* or DeBlock (WO 97/06267), one skilled in the art would not have been motivated to look for the longer gene sequence disclosed by Applicants. This longer gene sequence encodes the full-length, fully functional maize poly ADP-ribose polymerase protein, which has the amino acid sequence set forth in SEQ ID NO. 2. See also Mahajan *et al.*(1998) *Plant Physiol.*118:895-905, a copy of which was presented as cite no. 22 and

submitted with the Information Disclosure Statement filed June 18, 1999. As noted above, the full-length poly ADP-ribose polymerase protein comprises 11 amino acids not found in the putative maize poly ADP-ribose polymerase protein disclosed by Babiyachuk *et al.* The differences between the amino acid sequences disclosed by Applicants and by Babiyachuk *et al.* are significant when considering the structure-function relationship of the two proteins.

Based on its characteristic domain features, the full-length maize poly ADP-ribose polymerase protein disclosed by Applicants represents a classical-type poly ADP-ribose polymerase protein. The classical-type poly ADP-ribose polymerase protein has a molecular mass of 113-115 kDa and represents the major form of poly ADP-ribose polymerase activity in all higher organisms studied. It catalyzes addition of multiple ADP-ribose units to a variety of acceptor substrates under *in vivo* as well as *in vitro* conditions. As early as 1984, Zahradka and Ebisuzaki demonstrated that the classical-type poly ADP-ribose polymerase is a metalloenzyme that needs zinc for its activity. Two Zn^{++} ions are bond per poly ADP-ribose polymerase molecule, and the metal binding requires the N-terminal ~ 1/3 of the protein (Zahrdaka and Ebisuzaki (1984) *Eur. J. Biochem.* 142:503-509; an abstract of which is filed concurrently herewith as item 3 of Appendix A). Subsequently, this N-terminal region, known as the DNA binding domain (DBD), was shown to contain two zinc fingers of the type $C\text{-}aa_2\text{-}C\text{-}aa_{28\text{-}30}\text{-}H\text{-}Xaa_2\text{-}C$. These zinc fingers are conserved in all classical-type poly ADP-ribose polymerase proteins characterized to date, including the full-length maize poly ADP-ribose polymerase protein disclosed by Applicants.

This evolutionary conservation of the zinc fingers in the classical-type poly ADP-ribose polymerase molecule from all species, including maize, clearly indicates their functional relevance. In fact, a series of carefully conducted experiments involving site-directed mutagenesis of individual amino acids or deletion analysis of the zinc fingers clearly establishes the functional relevance of these zinc fingers in the biological activity of poly ADP-ribose polymerase. Thus, Ikejima *et al.* (1990) showed that deletion or mutation of the zinc fingers results in elimination or reduction in ability of poly ADP-

ribose polymerase to bind DNA, as well as substantial reduction in catalytic activity (*J. Biol. Chem.* 265(1990):21907-21913; submitted as cite no. 24 in the Information Disclosure Statement filed March 2, 2000). Furthermore, the residual DNA binding activity of the mutant poly ADP-ribose polymerase molecule is unrelated to DNA damage and does not result in activation of the catalytic function of the enzyme (Ikejima *et al.* (1990) *J. Biol. Chem.* 265:21907-21913).

In summary, the zinc fingers in the classical-type poly ADP-ribose polymerase protein are unique structurally as well as functionally. The C-aa_2-C-aa_{28-30}-H-Xaa_2-C type of structure is found only in poly ADP-ribose polymerase proteins, with the only exception being mammalian DNA ligase III, which is also involved in DNA repair. Zinc fingers recognize alteration in DNA structure rather than a specific sequence. Furthermore, the zinc fingers are required for binding of poly ADP-ribose polymerase to damaged DNA and the subsequent activation of the catalytic function of the protein.

Of the 11 amino acids missing in the putative maize poly ADP-ribose polymerase protein disclosed by Babiyachuk *et al.*, but present in Applicants' full-length maize poly ADP-ribose polymerase protein, 2 (residues 54-55 of SEQ ID NO.2) reside within a zinc-finger domain. Thus, the putative poly ADP-ribose polymerase protein disclosed by Babiyachuk *et al.* is missing one functional zinc finger. Indeed, in a 1998 reference directed to the putative maize poly ADP-ribose polymerase protein disclosed by Babiyachuk *et al.*, the co-authors state that "The first Zn-finger of ZAP1 was probably non-functional because it had the sequence CKSCxxxHASV that does not include the third cysteine residue" (Babiyachuk *et al.* (1998) *The Plant Journal* 15(5):635-645, page 636, column 2, lines 12-15 of the section entitled "*Zap is a Zn-finger-containing plant poly ADP-ribose polymerase*"; citation number 21 on the PTO Form 1449 filed with Applicants' June 18, 1999 Information Disclosure Statement). As used in this 1998 reference, ZAP1 refers to the N-terminal portion of the putative maize poly ADP-ribose polymerase protein disclosed by Babiyachuk *et al.*

Thus, the putative maize poly ADP-ribose polymerase protein disclosed by Babiyachuk *et al.* does not comprise 2 amino acids that reside within a critical structure of the fully functional protein. Prior to Applicants' disclosure, these residues were not

known, and hence the fully functional, classical-type maize poly ADP-ribose polymerase protein was not known.

Applicants also respectfully submit that DeBlock (WO 97/06267), either alone or in combination with Babiyachuk *et al.*, does not teach, nor render obvious, Applicants' invention. Neither of these references teaches the recombinant methods of the present invention, i.e., transformation with the nucleotide sequences encompassed by amended claim 1. Furthermore, Babiyachuk *et al.* does not disclose the sequences encompassed by amended claim 1. As noted previously, claims 2-23 as amended all require knowledge or use of the full-length sequences recited in amended claim 1.

Thus, the present invention as a whole could not have been practiced without Applicants' disclosure of the full-length maize poly ADP-ribose polymerase nucleotide and amino acid sequences. As such, the rejection under 35 U.S.C. §103 should be withdrawn.

Presentation of New Claims

New claims 26-33 are presented as noted herein above. Support for these claims resides throughout the specification. Applicants respectfully submit that these claims are definite, fully described in and enabled by the specification, and are not taught or suggested by the references previously cited by the Examiner.

CONCLUSION

In view of the aforementioned amendments and remarks, Applicants respectfully submit that the rejections of the claims under 35 U.S.C. §§102, 103, and 112 are overcome. Accordingly, Applicants submit that this application is now in condition for allowance. Early notice to this effect is solicited.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to

allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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Lynda J. Pixley June 26, 2002
Signature Date

Version with Markings to Show Changes Made:

Please cancel claim 16 without prejudice to or disclaimer of the subject matter contained therein.

Please amend claims 1-9, 11, 12, 15, 18, and 21 to read as follows:

1. (Amended) An isolated DNA molecule comprising a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence encoding a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 2[,];

b) the nucleotide sequence set forth in SEQ ID NO. 1[,]; and

c) a nucleotide sequence that [corresponds to an]is antisense to the full-length sequence [for the nucleotide sequence]set forth in SEQ ID NO. 1[,

d) a nucleotide sequence encoding the C-terminal domain of a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 2,

e) a nucleotide sequence encoding the C-terminal domain of a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 4,

f) the nucleotide sequence set forth in SEQ ID NO. 3,

g) a nucleotide sequence that hybridizes to any one of the nucleotide sequence of a) - f) under stringent conditions].

2. (Amended) A chimeric [gene]nucleic acid sequence comprising a promoter capable of driving expression of a [gene]nucleic acid sequence in a plant cell operably linked to a nucleotide sequence of claim 1.

3. (Amended) The chimeric [gene]nucleic acid sequence of claim 2, wherein the nucleotide sequence encodes a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 2.

4. (Amended) The chimeric [gene]nucleic acid sequence of claim 3, wherein said [coding]nucleotide sequence is the nucleotide sequence set forth in SEQ ID NO. 1.

5. (Amended) A vector comprising the chimeric [gene]nucleic acid sequence of claim 4.

6. (Amended) A plant cell transformed with the chimeric [gene]nucleic acid sequence of claim [5]4.

7. (Amended) A transformed plant comprising the chimeric [gene]nucleic acid sequence of claim 4.

8. (Amended) The chimeric [gene]nucleic acid sequence of claim 2, wherein the nucleotide sequence is [an]antisense to the full-length sequence [for a plant poly ADP-ribose polymerase]set forth in SEQ ID NO.1.

9. (Amended) A vector comprising the chimeric [gene]nucleic acid sequence of claim 8.

11. (Amended) A transformed plant comprising the chimeric [gene]nucleic acid sequence of claim 8.

12. (Amended) A transformed plant having incorporated into its genome a DNA molecule, said molecule comprising a promoter capable of driving expression of a [gene]nucleic acid sequence in a plant cell operably linked to a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence encoding a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 2[,];

b) the nucleotide sequence set forth in SEQ ID NO. 1[,]; and

c) a nucleotide sequence that [corresponds to an]is antisense to the full-length sequence [for the nucleotide sequence]set forth in SEQ ID NO. 1[,

d) a nucleotide sequence encoding the C-terminal domain of a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 2,

e) a nucleotide sequence encoding the C-terminal domain of a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 4,

f) the nucleotide sequence set forth in SEQ ID NO. 3,

g) a nucleotide sequence that hybridizes to the nucleotide sequence of a) - f) under stringent conditions].

15. (Amended) The transformed plant of claim 12, wherein the nucleotide sequence is [an]antisense to the full-length sequence [for a plant poly ADP-ribose polymerase]set forth in SEQ ID NO.1.

18. (Amended) The transformed plant of claim [13]12, wherein said plant is a monocot.

21. (Amended) A method for modulating the metabolic state of a plant cell, said method comprising transforming said plant with a DNA construct, said construct comprising a promoter that drives expression in a plant cell operably linked with a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence encoding a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 2[,];

b) the nucleotide sequence set forth in SEQ ID NO. 1[,]; and

c) a nucleotide sequence that [corresponds to an]is antisense to the full-length sequence [for the nucleotide sequence]set forth in SEQ ID NO. 1[,

d) a nucleotide sequence encoding the C-terminal domain of a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 2,

e) a nucleotide sequence encoding the C-terminal domain of a poly ADP-ribose polymerase having the amino acid sequence set forth in SEQ ID NO. 4,

f) the nucleotide sequence set forth in SEQ ID NO. 3,

g) a nucleotide sequence that hybridizes to the nucleotide sequence of a) - f) under stringent conditions].

APPENDIX A
Item 1

AttyDktNo. 5718-34 (35718/174234)
Appl. No. 09/236,995
Filed January 26, 1999

Record 4 of 8 in Biological Abstracts 1998/07-1998/12
TI: Regulation of the expression or recruitment of components of the DNA synthesome by poly(ADP-Ribose) polymerase.
AU: Simbulan-Rosenthal-Cynthia-M; Rosenthal-Dean-S; Boulares-A-Hamid; Hickey-Robert-J; Malkas-Linda-H; Coll-Jennifer-M; Smulson-Mark-E {a}
SO: Biochemistry-. June 30, 1998; 37 (26) 9363-9370.
PY: 1998
LA: English
AB: Poly(ADP-ribose) polymerase (PARP) is a component of the multiprotein DNA replication complex (MRC, DNA synthesome) that catalyzes replication of viral DNA in vitro. PARP poly(ADPribosyl)ates 15 of the apprx40 proteins of the MRC, including DNA polymerase alpha (DNA pol alpha), DNA topoisomerase I (topo I), and proliferating-cell nuclear antigen (PCNA). Although about equal amounts of MRC-complexed and free forms of PCNA were detected by immunoblot analysis of HeLa cell extracts, only the complexed form was poly(ADP-ribosyl)ated, suggesting that poly(ADP-ribosyl)ation of PCNA may regulate its function within the MRC. NAD inhibited the activity of DNA pol delta in the MRC in a dose-dependent manner, whereas the PARP inhibitor, 3-AB, reversed this inhibitory effect. The roles of PARP in modulating the composition and enzyme activities of the DNA synthesome were further investigated by characterizing the complex purified from 3T3-L1 cells before and 24 h after induction of a round of DNA replication required for differentiation of these cells; at the latter time point, apprx95% of the cells are in S phase and exhibit a transient peak of PARP expression. The MRC was also purified from similarly treated 3T3-L1 cells depleted of PARP by antisense RNA expression; these cells do not undergo DNA replication nor terminal differentiation. Both PARP protein and activity and essentially all of the DNA pol alpha and delta activities exclusively cosedimented with the MRC fractions from S phase control cells, and were not detected in the MRC fractions from PARP-antisense or uninduced control cells. Immunoblot analysis further revealed that, although PCNA and topo I were present in total extracts from both control and PARP-antisense cells, they were present in the MRC fraction only from induced control cells, indicating that PARP may play a role in their assembly into an active DNA synthesome. In contrast, expression of DNA pol alpha, DNA primase, and RPA was down-regulated in PARP-antisense cells, suggesting that PARP may be involved in the expression of these proteins. Depletion of PARP also prevented induction of the expression of the transcription factor E2F-1, which positively regulates transcription of the DNA pol alpha and PCNA genes; thus, PARP may be necessary for expression of these genes when quiescent cells are stimulated to proliferate.
AN: 199800346892
UD: 19980707

APPENDIX A
Item 2

AttyDktNo. 5718-34 (35718/174234)
Appl. No. 09/236,995
Filed January 26, 1999

Record 8 of 8 in Biological Abstracts 1999/07-1999/12
TI: Involvement of PARP and poly(ADP-ribosyl)ation in the early stages of apoptosis and DNA replication.
AU: Simbulan-Rosenthal-Cynthia-Marie; Rosenthal-Dean-S; Iyer-Sudha; Boulares-Hamid; Smulson-Mark-E {a}
SO: Molecular-and-Cellular-Biochemistry. March, 1999; 193 (1-2): 137-148.
PY: 1999
LA: English
AB: We have focused on the roles of PARP and poly(ADP-ribosyl)ation early in apoptosis, as well as during the early stages of differentiation-linked DNA replication. In both nuclear processes, a transient burst of PAR synthesis and PARP expression occurs early, prior to internucleosomal DNA cleavage before commitment to apoptosis as well as at the round of DNA replication prior to the onset of terminal differentiation. In intact human osteosarcoma cells undergoing spontaneous apoptosis, both PARP and PAR decreased after this early peak, concomitant with the inactivation and cleavage of PARP by caspase-3 and the onset of substantial DNA and nuclear fragmentation. Whereas 3T3-L1, osteosarcoma cells, and immortalized PARP +/+ fibroblasts exhibited this early burst of PAR synthesis during Fas-mediated apoptosis, neither PARP-depleted 3T3-L1 **PARP-antisense** cells nor PARP -/- fibroblasts showed this response. Consequently, whereas control cells progressed into apoptosis, as indicated by induction of caspase-3-like PARP-cleavage activity, **PARP-antisense** cells and PARP -/- fibroblasts did not, indicating a requirement for PARP and poly(ADP-ribosyl)ation of nuclear proteins at an early reversible stage of apoptosis. In parallel experiments, a transient increase in PARP expression and activity were also noted in 3T3-L1 preadipocytes 24 h after induction of differentiation, a stage at which apprx95% of the cells were in S-phase, but not in **PARP-depleted antisense** cells, which were consequently unable to complete the round of DNA replication required for differentiation. PARP, a component of the multiprotein DNA replication complex (MRC) that catalyzes viral DNA replication in vitro, poly(ADP-ribosyl)ates 15 of apprx40 MRC proteins, including DNA pol alpha, DNA topo I, and PCNA. Depletion of endogenous PARP by antisense RNA expression in 3T3-L1 cells results in MRCs devoid of any DNA pol alpha and DNA pol delta activities. Surprisingly, there was no new expression of PCNA and DNA pol alpha, as well as the transcription factor E2F-1 in **PARP-antisense** cells during entry into S-phase, suggesting that PARP may play a role in the expression of these proteins, perhaps by interacting with a site in the promoters for these genes.
AN: 199900170847
UD: 19990630